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Review Article

Gap Junctions in Ischemia-Related Ventricular Arrhythmia

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Cardiovascular diseases are the leading cause of death worldwide, with ischemic heart disease being the most common entity. Ventricular arrhythmia is one of the complications of ischemia that can result in sudden cardiac death. One of the underlying mechanisms of ischemia induced arrhythmia is closure of cardiac myocytes gap junctions, where gap junctions are channel-like structure between cells that allow passage of molecules and electrical current. During ischemia, gap junctions close incompletely, creating tissue impedance heterogeneity and conduction slowing, which provide substrate for ventricular arrhythmia. Conditions where gap junctions structure is altered, such as in heart failure, is associated with increased vulnerability of ischemia-induced ventricular arrhythmia.

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Keywords: Ischemic ventricle arrhythmias, gap junction

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Tinjauan Pustaka

Gap Junctions pada Ischemia-Related Ventrikel Aritmia

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Penyakit kardiovaskular merupakan penyebab utama kematian di dunia, dengan penyakit jantung iskemik sebagai yang tersering. Salah satu komplikasi iskemia adalah aritmia ventrikular yang dapat mengakibatkan kematian jantung mendadak. Mekanisme yang mendasari terjadinya aritmia yang diinduksi-iskemia antara lain adalah menutupnya *gap junction* –suatu struktur antar-sel yang memungkinkan aliran molekul dan arus listrik– di sel otot jantung. Saat iskemia, *gap junction* tidak menutup sempurna sehingga tercipta keragaman impedansi (tahanan) jaringan dan perlambatan konduksi (hantaran), yang menyediakan substrat bagi aritmia ventrikular. Kondisi yang mengakibatkan berubahnya struktur *gap junction,* seperti yang terjadi pada gagal jantung, membuat jantung lebih rentan untuk mengalami aritmia ventrikular yang diinduksi-iskemia.

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Kata kunci: ventrikel aritmia iskemik, gap junction

Cardiovascular diseases are the leading cause of death, both in developed and developing countries, with ischemic heart disease being the most frequent clinical entity.[1] Ventricular arrhythmia is one of fatal complications of ischemia that can result in sudden cardiac death.[2] One of the underlying mechanisms of ischemia-induced arrhythmia is the closure of cardiac myocytes gap junctions.[3,4] Gap junctions are regions of close apposition between cells with a narrow (1-2 nm) gap between the cell membranes, which allow passage of small molecules and electric current.[3] These structures present in many tissues, one of it being the cardiac muscles, and are involved in a number of biologic functions such as electrical conduction, embryogenesis and cell growth.[4]

Revel and Karnovsky first used the term 'gap junction' in 1967 when showing that there is a measurable gap at the junction of intercalated discs in mouse heart (Figure 1).[5] Tangential section of the junctions showed an extraordinarily regular pattern of hexagonally packed structures, with each hexagon appears like a hollow-prism, suggesting a channel-type structure that connects cells with close membrane apposition.

After their discoveries, gap junctions have been extensively studied with various and more advanced methods such as x-ray diffraction and atomic force microscopy. Perkins, et al, for example, constructed three-dimensional images of gap junctions using electron crystallographic method (Figure 2).[6]

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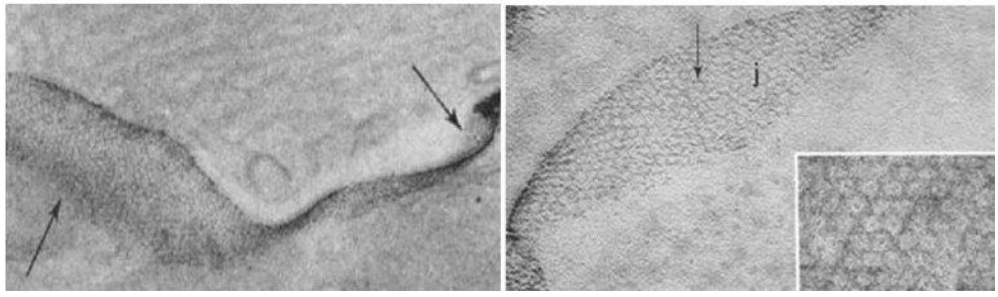

Figure 1A. Upper arrow displayed the regions of close membrane apposition; lower arrow is the same structure in tangential view, showed hexagonally packed structures in the junctional area. **B.** Further magnification showed a regular pattern of hexagons (j and inset) that have an electron-opaque core (arrow), and an electron-transparent wall around 30-40 Å thick (inset). (Figure 3A x100,000, 3B x210,000, inset x420,000). Taken from reference.[5]

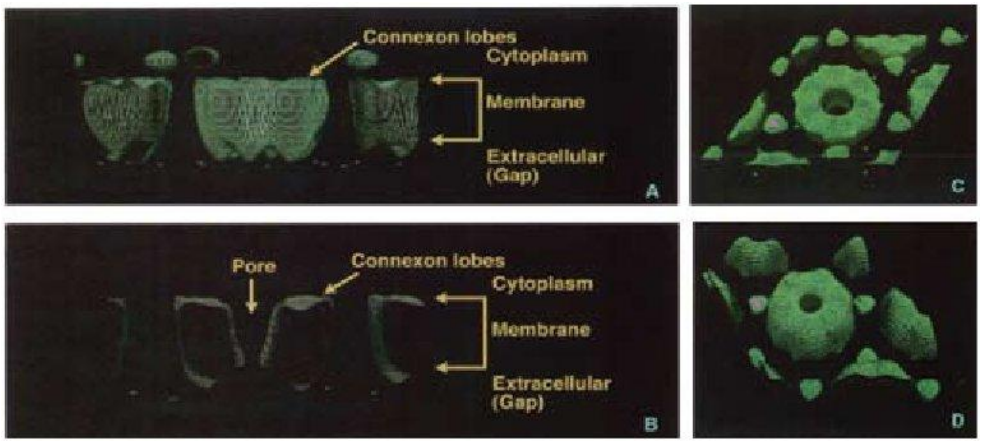

Figure 2. A central connexon and parts of the surrounding connexons at the edges of a cell. **A.** A side view of the entire map showing the connexon, cytoplasm and extracellular side. **B.** A view of the central portion cut parallel to the x-axis and emphasizes how the channel tapers toward the extracellular side. **C.** A view looking down the cytoplasmic portion of the structure, at a small angle from the six-fold axis. **D.** Connexon reconstruction viewed looking down the extracellular side at a small angle from the six-fold axis, located at the center of the connexon. Taken from reference[6]

Gap junctions structure

In myocardium, gap junctions provide pathways for direct cell-to-cell communication between adjacent myocytes, facilitating rapid conduction of depolarization to carry out highly-coordinated contraction. In cardiac myocytes, gap junctions are found at the intercalated disk. Gap junctions are formed from proteins called connexin that span the membrane lipid bilayer of myocytes. Six connexins oligomerize or group to form hexameric half (or hemi) channels called connexon. Two connexons in adjacent cells then meet head to head across the extracellular space to form a complete channel, i.e. one gap junction channel (Figure 3).[4,7]

There are currently 20 different connexin isotypes known to exist in the human genome.[8] Connexins are commonly classified according to the molecular weight of the protein predicted from its DNA sequence; e.g. connexin 43 has a predicted molecular weight of 43 kD. Each isotype is expressed in selected tissues, and most tissues express more than one type of connexins. The functional importance is not fully understood, but each of the isotype may have specific properties that are necessary for the tissues where it is expressed.[8]

Multiple connexins are coexpressed in many cardiovascular tissues. The cardiac connexins include connexin43 (Cx43), connexin40 (Cx40), and connexin45 (Cx45), with Cx43 being the most abundant

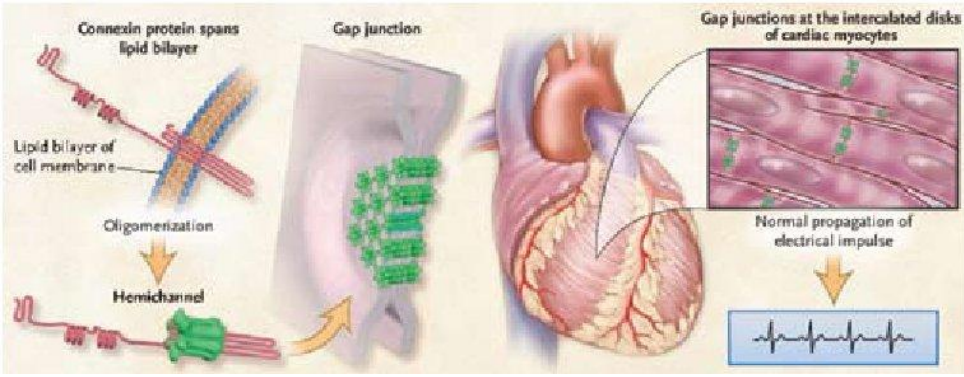

Figure 3. Schematic illustrations of gap junctions and their supra- and infra-structures found in cardiac myocytes that have roles in conduction of impulse. Taken from reference[7]

and the one most extensively studied. Another connexin (Cx37) is found in blood vessels. Additional connexins may also be present in cardiovascular cells, but few convincing data have been published.[9] The cardiovascular connexins show distinct patterns of distribution and expression, which contribute to the different conduction properties of distinct regions of myocardium and various portions of the conduction systems.[9]

Functions of gap junctions

In the heart, current flows through GJ synchronizes pacemaking activity and coordinates the spread of contractile waves that generate myocardial contraction. Cx43 knockout mice have severely disturbed cardiac rhythm.[10] In the vasculature, GJ synchronize endothelial responses, modulate vascular tone, and may even provide a pathway for direct endothelial-smooth muscle communication.[11]

Cx43 is the principal ventricular electrical coupling protein. It is shown that in Cx43+/- genetically-engineered mice, which only expressed 50% Cx43, there is a modest (~25% to 30%) slowing of ventricular epicardial conduction, with atrial conduction remains normal. Mice that do not have cardiac Cx43 (i.e. conditional Cx43-/-) experience malignant ventricular tachycardia, fibrillation, and sudden cardiac death in young adulthood.[12] Cx40 is the major coupling protein in atrial myocardium. Cx40-null mice have normal ventricular conduction velocity, but they exhibit atrial conduction disturbances and atrial arrhythmias.[13]

These observations suggest that Cx43 and Cx40 are chamber-specific molecular determinants of intercellular coupling in atrial and ventricular muscle. The pathophysiologic significance is that altered expression of connexin subtypes, particularly in spatially heterogeneous patterns such as in ischemia, may produce substrates for discontinuous conduction and arrhythmogenesis.

Gap junctions closure during ischemia

High degree of coupling between myocytes carries the risk that when the heart is injured, chemical mediators will spread and increase the damage to the heart. Under ischemic injury, apoptotic signals may spread from cell to cell, increasing area of cell death. Such spread of damage from the directly injured cells to their neighbors has been termed 'bystander cell' killing.[14] Thus, it is potentially beneficial for cardiac myocytes to uncouple rapidly from injured cells by closing of gap junction channels under this condition. However, there is evidence that the process may be incomplete, giving substrate for arrhythmogenesis and still allowing passage of cytotoxic molecules to kill bystanders. Changes in connexin expression and redistribution of gap junctions at the infarct border zone have been convincingly linked to conduction disturbances that may promote reentrant arrhythmia.[15,16] Uncoupling during acute ischemia involves complex changes in connexin function and distribution that are presumably mediated by multiple stress activated pathways. During the process, Cx43 undergoes marked dephos-

phorylation and redistribution from gap junction to intracellular sites.[16]

Several factors associated with ischemia-related uncoupling have been identified. Increase of diastolic cytoplasmatic [Ca^{2+}] is associated with gap junctional uncoupling. Ischemia-induced intracellular acidification decreases gap junctional conductance and makes gap junctions more sensitive to increased [Ca^{2+}]$_i$.[17] Ischemia causes accumulation of lysophosphoglycerides and arachidonic acid metabolites in the intercalated disks,[18] and induces release of catecholamines[19] which decrease gap junctional conductance.

Ventricular arrhythmia during acute myocardial ischemia

Acute myocardial ischemia is the major cause of sudden cardiac death, which in most cases is due to ventricular arrhythmias, particularly ventricular fibrillation (VF). There are two phases of ventricular arrhythmias during the first hour of ischemia, i.e. 1A (immediate) and 1B (delayed) phase.[20] Phase 1A lasts from 2 to 8 min of ischemia in the dog. After a relative arrhythmia-free interval, 1B phase occurs from 15 to 45 min of coronary occlusion.[21] It is unclear whether a similar distribution of ischemia-induced arrhythmias exists in human, as it is extremely difficult to study. The 1B phase of arrhythmias coincides with the increase of tissue impedance (Figure 4) and therefore was thought to be causally related with gap junctional uncoupling.[21]

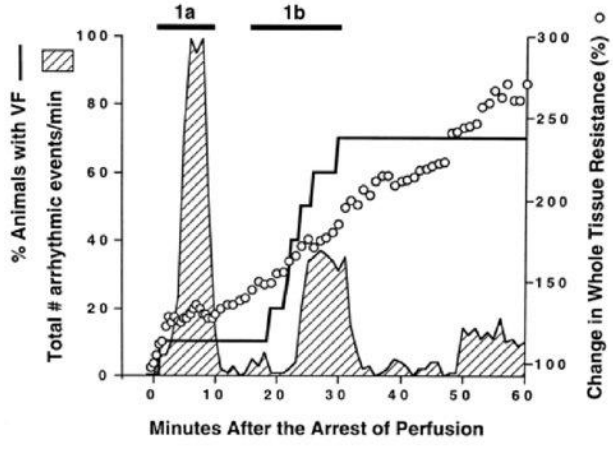

Figure 4. Plot showing the percentage of pigs with VF during the 60-minute ischemic period (solid line, left axis). On the same axis, the total number of arrhythmic events (PVCs, short runs of VT, and the occurrence of VF) per 1-minute interval is represented by the hatched area. Open circles indicate mean percent change in tissue resistance for nine experiments after the arrest of coronary flow; solid bars, the Ia and Ib phases of ventricular arrhythmias.[21]

Reentry is the underlying electrophysiological mechanism of ventricular fibrillation.[22] The requirements for reentrant activation are a region of unidirectional block and (regionally) slow-enough conduction velocity to allow the activation impulse to travel around the zone of block. For an arrhythmia to occur, both a suitable substrate (the preexisting circumstances that allow maintenance of the arrhythmia) and a trigger (the event that sets off the arrhythmia within the substrate) need to be present.[23] The exact mechanisms on how gap junctional uncoupling causes conduction slowing and arrhythmias in the regionally ischemic heart is not completely understood.

During acute myocardial ischemia, the conditions necessary for the initiation of reentry, both trigger and substrate, occur in concert and, indeed, ventricular fibrillation is often encountered.[22] In the course of ischemia, gap junctions close (i.e. uncouple), which contribute to the initiation of reentrant activation and ventricular fibrillation by the creation of tissue impedance heterogeneities and conduction slowing.[24] Arrhythmogenic triggers exist most often from a timely administrated or spontaneous premature ventricular complexes from mechanical stretch exerted by the viable myocardium on the ischemic border during the 1B phase.[22]

Tissue impedance

Tissue impedance is the composite measure of resistance and reactance and an indirect measure for intercellular coupling. *Tissue impedance heterogeneity* is a condition where the impedance of a particular tissue in a given time is heterogenous, which is due to the evolution of a surviving subepicardial and subendocardial layer. It has been established that a rim of subepicardial and subendocardial tissue survives ischemia and infarction and that intramural sites become electrically inexcitable during prolonged ischemia, whereas subepicardial cells remain activated (Figure 5).[21] The increase in tissue impedance was significantly smaller in the ischemic border zone than in the central zone, although the time course of rise was identical.[25] Hence, ischemic and nonischemic myocardium interdigitate at the ischemic

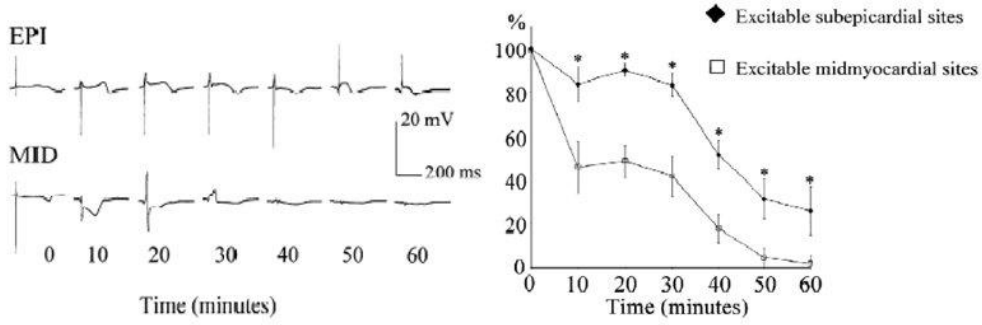

Figure 5. A. Bipolar electrograms from subepicardium (EPI) and midmyocardium (MID) during 60 min of ischemia [numbers indicate time (min)]. Note that, after 60 minutes, the subepicardial electrogram remains essentially unchanged whereas midmyocardial excitability ceased after 30 minutes. **B.** Percentage of excitable subepicardial and midmyocardial sites during 60 min of ischemia (mean6S.E.M.). Asterisks indicate P<0.01, subepicardium versus midmyocardium. Taken from reference[25]

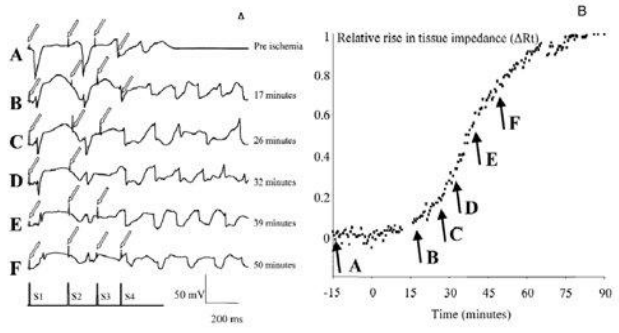

Figure 6. A. Unipolar electrograms of premature stimulation in a regionally ischemic pig heart. Arrows denote stimulation artifacts. The number of PVC decreased from three to one at 32 min of ischemia, after which more stimuli are needed to induce VF. **B:** Corresponding rise in tissue impedance in the same experiment as in A. Letters indicate when electrograms in A were recorded. Taken from reference[25]

border, where ischemic tissue impedance increases, and nonischemic tissue impedance remains normal.

Several observations support the role of heterogeneity in ischemia related arrhythmia. De Groot, et al. showed that VF could be induced with programmed stimulation between 14 and 53 min of ischemia. Thereafter, the same induction protocol failed to induce VF.[25] Figure 6 shows electrograms of VF inducibility in an isolated regionally ischemic pig heart. The number of PVCs to induce VF decreases from three during control to one at 32 minutes of ischemia, after which more PVC are required to induce VF. The right panel shows the rise in tissue impedance.[25] Thus, by providing the necessary triggers for VF, these experiments indicate that the substrate for VF is evolving during the 1B phase, and that the optimum substrate is present when uncoupling is moderate.

Conduction slowing

In normoxic condition the relation between gap junctional uncoupling and decreased conduction velocity appears clear. Jongsma and Wilders confirmed that, under nonischemic conditions, approximately 90% decrease of gap junctions is required to decrease conduction velocity by 50%.[26] During ischemia additional factors contribute to conduction slowing that appears to be caused by electrotonic interaction between the large mass of depolarized–dying–intramural cells and non ischemic subepicardial and subendocardial cells.[27]

Preexisting conditions and gap junctional coupling

In Cx43 +/- mice, significantly more spontaneous and induced arrhythmias were observed during 1 hour of regional ischemia.[28] Both spontaneous and induced VTs are more frequent in the Cx43 +/- animals. The

hearts of these animals were morphologically normal and conduction velocity under normoxic circumstances was only marginally reduced. Therefore, it was hypothesized that the increased arrhythmogenicity resulted from the interplay between acute ischemia and the genetic background of reduced gap junctional coupling.[28] Heart failure patients are more prone to arrhythmia and the incidence is more severe due to the preexisting morphological changes, where the number of gap junctions decreases and lateralization occurs.[29] Derksen, et al demonstrated pathologic conduction curves occur in hearts with interstitial fibrosis which is associated with high vulnerability of VF. [30]

Conclusions

In order to work properly, the heart needs high level coordination between cells. This coordination is facilitated by gap junction which allow free movement of electrical and ionic current among cells. In ischemic condition, uncoupling of gap junction channels occurs as a way to protect adjacent cells from dying. Incomplete, moderate level of gap junctional uncoupling provide substrates for ventricular arrhythmias due to increase in tissue impedance and conduction slowing. Conditions where number of gap junctions decreased such as in failing heart are more prone to ventricular arrhythmias.

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